Interaction of T-2 Toxin with *Salmonella* Infections of Chickens[1,2]

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ABSTRACT A significant ($P < 0.05$) interaction resulting in increased mortality occurred in chickens fed T-2 toxin (16 μg./g. of diet) and infected with either *Salmonella worthington*, *S. thompson*, *S. derby*, or *S. typhimurium* var. *copenhagen*, all species that cause paratyphoid. No interaction on growth rate or relative size of the bursa of Fabricius occurred, although T-2 toxin alone caused a significant ($P < 0.05$) regression of that organ. The spleen size relative to the body weight was decreased ($P < 0.05$) by T-2 toxin and increased ($P < 0.05$) by the *Salmonella* infections. Interactions were observed on spleen size between the toxin and *S. thompson* ($P < 0.05$) and *S. derby* ($P < 0.10$). Total serum proteins were not affected by T-2 toxin or *Salmonella* infections. Agglutinins were formed in response to the infections, but the titers were unaltered by T-2 toxin.

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INTRODUCTION

THE question of possible interactions between mycotoxins and infectious disease agents has been important to animal husbandry and health ever since one of the first reports of aflatoxicosis (Siller and Ostler, 1961) described the isolation of *Salmonella* from the internal organs of turkeys during field outbreaks of the disease. T-2 toxin is a potent mycotoxin produced by several species of the genus *Fusarium* (Bamburg *et al.*, 1969) and its chemical structure is 4,15-diacetoxy-8-(3-methylbutyryloxy)-12,13-expoxy-Δ^9-tricothecen-3-ol (Bamburg *et al.*, 1968). Like most 12,13-epoxy-Δ^9-tricothecen compounds it elicits a severe inflammatory reaction in animals.

Dietary T-2 toxin in trout induces rapid sloughing of intestinal mucosa. In rats, it causes dermal necroses and inflammation (Marasas *et al.*, 1969), and in chickens severe dose-related oral lesions similar to the third or necrotic stage of alimentary toxic aleukia, a nutritional toxicosis of humans (Wyatt *et al.*, 1972b). Oral lesions in chickens appear characteristic enough to aid in the diagnosis of T-2 toxicosis (Wyatt *et al.*, 1972a). In addition, dietary T-2 toxin reduced growth rate and decreased size of the spleen and bursa of Fabricius in chickens (Wyatt *et al.*, 1973). Because T-2 toxin has a pronounced inflammatory action on the gastrointestinal tract and because *Salmonella* is an intestinal pathogen, we decided to explore the interactions between these two factors important to poultry health.

MATERIALS AND METHODS

Production of T-2 Toxin. *Fusarium tricinctum* NRRL 3299 was grown on white corn grits (Burmeister, 1971). The toxin was extracted and purified by the method of Burmeister (1971) to yield a crystalline product melting at 148 to 150° C.

Animal Husbandry. One-day-old male broiler chickens (Cobb × Cobb) were pur-

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chased and housed in metal brooding batteries. Since a brooding source of heat was not desired, the chicks were maintained at an ambient temperature of 30° C. Feed and water were available *ad libitum*. Feed consisted of a commercial broiler-starter diet free of all medication.

Administration of Toxin. T-2 toxicosis was induced by incorporating into small portions of the diet known amounts of crystalline T-2 toxin dissolved in 50% (v./v.) aqueous ethanol. The portions of feed containing T-2 toxin were dried at 100° C. to evaporate the ethanol before being mixed thoroughly into the remainder of the feed. Chickens were fed experimental diets from hatching until the experiment was terminated at 3 weeks.

Bacteria. *Salmonella typhimurium* var. *copenhagen*, *S. thompson*, *S. derby*, and *S. worthington*, used in this study, had been isolated from paratyphoid outbreaks in young chickens. The bacteria were identified by the Center for Disease Control (Atlanta, Ga.). Cultures were prepared by inoculating veal infusion broth (Difco) with a fresh inoculum of each species and incubating at 37° C. for 18 hours.

Experimental Design. A total of 400 birds was used in this experiment, and the experimental design was completely randomized. Half the birds were fed a toxin-free diet. The other half received the same diet but containing 16 μg. of T-2 toxin per g. At one week of age, four groups of ten birds fed each diet were inoculated orally with 1.0 ml. of an 18-hour culture containing 1.0×10^8 cells of *Salmonella*. Body weights were measured and mortality rates were recorded at the end of the experiment. At three weeks of age, blood samples from the survivors were taken by cardiac puncture. The sera from samples were tested for agglutinins against the homologous *Salmonella* species using antigen prepared as described by Yamamoto *et al.* (1962). Sera were combined on a group basis, and all agglutinations were run on the pooled sera. Total serum protein was determined by a biuret method (Gornall *et al.*, 1949). The birds were killed by cervical dislocation. Immediately after death, the spleen and bursa of Fabricius were excised, blotted, and weighed. The size of the organs was expressed relative to the body weight of the birds from which they were excised. The data were subjected to factorial analyses of variance in which an F-ratio was the measure of significance, except for mortality data that were analyzed according to a test for the significance of differences between two proportions (Bruning and Kintz, 1968).

RESULTS

Dietary T-2 toxin affected the mortality rate of chickens with paratyphoid infections (Table 1). Neither factor alone caused mortality, but the two in combination produced significant ($P < 0.05$) mortality. This interaction on mortality was not accompanied by an interaction on growth rate (Table 2). However, T-2 toxin, *S. derby*, and *S. typhimurium* var. *copenhagen* individually decreased the growth rate significantly ($P < 0.01$).

In Table 3 are recorded the effect of T-2

TABLE 1.—*Effect of T-2 toxin and* Salmonella *on mortality in chickens*

Organism	T-2 toxin (μg./g.)	
	0	16
Control	0/40[1]	0/40
S. worthington	0/40	2/40[a]
S. thompson	0/40	5/40[a]
S. derby	0/40	3/40[a]
S. typhimurium var. *copenhagen*	0/40	7/40[a]

[1] Mortality is expressed as number of deaths per number of birds in a treatment.
[a] These values differ ($P < 0.05$) from the corresponding control values.

TABLE 2.—*Effect of T-2 toxin and* Salmonella *infections on the body weight of chickens*

Organism	T-2 toxin (μg./g.) 0	16
Control	422[1]	259
S. worthington	419	238
S. thompson	411	246
S. derby	404	218
S. typhimurium var. *copenhagen*	405	218

[1]Each value is the mean body weight in grams of 4 groups of 10 birds. A factorial analysis of variance revealed that only T-2 toxin had a significant ($P < 0.01$) effect and there was no interaction.

TABLE 3.—*Effect of T-2 toxin and* Salmonella *infections on relative organ weights of chickens*

Organism	T-2 toxin (μg./g.)	Organ weight (g./100 g. body weight)[1] Spleen	Bursa
Control	0	0.116[a]	0.390[a]
S. worthington	0	0.130[b]	0.361[a]
S. thompson	0	0.139[b]	0.376[a]
S. derby	0	0.140[b]	0.385[a]
S. typhimurium var. *copenhagen*	0	0.131[b]	0.378[a]
Control	16	0.080[c]	0.292[b]
S. worthington	16	0.102[d]	0.295[b]
S. thompson	16	0.090[d]	0.287[b]
S. derby	16	0.096[d]	0.288[b]
S. typhimurium var. *copenhagen*	16	0.101[d]	0.287[b]

[1]Each value is the mean of 4 groups of 10 birds.
[a,b,c,d]Values in a column with different superscripts differ ($P < 0.05$) significantly.

toxin and *Salmonella* infections on the size of the spleen and bursa of Fabricius. The bursa did not show an interaction between *Salmonella* and T-2 toxin although T-2 toxin by itself caused a significant regression of the organ. The spleen size, however, was decreased ($P < 0.05$) by the toxin and increased ($P < 0.05$) by the *Salmonella* infections. In addition, the analysis of variance revealed interactions on the spleen between the toxin and *S. thompson* ($P < 0.05$) and *S. derby* ($P < 0.10$). The responses of total serum proteins and anti-*Salmonella* agglutinins to paratyphoid and T-2 toxin are seen from Table 4. The total serum proteins were not affected by either factor. As expected, agglutinins were formed in response to the infections, but titers were not altered by dietary T-2 toxin.

TABLE 4.—*Effect of T-2 toxin and* Salmonella *on total serum proteins and agglutinin titers in chickens*

Organism	T-2 toxin (μg./g.)	Total serum protein (g./100 ml.)	Agglutinin titer[1]
Control	0	3.21	0
S. worthington	0	3.26	80
S. thompson	0	3.06	80
S. derby	0	3.15	160
S. typhimurium var. *copenhagen*	0	3.00	80
Control	16	3.02	0
S. worthington	16	2.98	80
S. thompson	16	2.84	80
S. derby	16	3.04	160
S. typhimurium	16	3.09	80

[1]The titers are expressed as the reciprocal of the highest serum dilution which gave agglutination.

DISCUSSION

The interaction demonstrated between T-2 toxin and paratyphoid infections, which manifests itself as increased mortality, has important implications for the poultry industry. Not only must mycotoxins be considered for their unique effects, but they must be evaluated for the likelihood of interaction with infectious agents and environmental stresses commonly encountered in poultry husbandry. Such interactions have been demonstrated previously with aflatoxin (Hamilton and Harris, 1971) and ochratoxin (Huff and Hamilton, unpublished results). Implicit to these interactions are the existence of syndromes of apparently unknown etiology and epidemiology and the difficulty of establishing "no effect" levels of mycotoxins under field conditions. Consider, for example, that paratyphoid interacts with low tem-

peratures (Thaxton *et al.*, 1974) as well as T-2 toxin.

The mechanism whereby T-2 toxin and paratyphoid infections interact to cause mortality cannot be deduced from the present study, but some speculations can be made. The well-demonstrated inflammatory and irritant action of T-2 toxin toward the gastrointestinal tract (Marasas *et al.*, 1969; Wyatt *et al.*, 1973) suggests that the natural barriers to invasion by the intestinal pathogen *Salmonella* (Perry *et al.*, 1972) may be more readily breached during T-2 toxicosis. The resulting bacteremia would normally be combatted by the phagocytic cells, but the smaller spleen caused by T-2 toxin and the negative interaction on spleen size by T-2 toxin and *S. derby* or *S. thompson* (Table 3) suggest that phagocytic cells of the reticuloendothelial system are impaired. This suggestion is also supported by the failure to find increased anti-*Salmonella* agglutinins during T-2 toxicosis (Table 4) which would be expected if the invading bacteria were encountering normal defenses. Another factor that may play a role is the generalized stress caused by T-2 toxin as indicated by a regressed bursa of Fabricius (Table 3). These considerations suggest several experimental approaches to the many puzzling facets of mycotoxicoses.

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